Docket No.: 17563/004001
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Kazuhiro Fukae

22511
PATENT TRADEMARK OFFICE

Application No.: 10/544,212

Confirmation No.: 8443

Filed: August 2, 2005

Art Unit: 1651

For: PROCESS FOR PRODUCING SUGAR CHAIN ASPARAGINE DERIVATIVE

Examiner: K. Ariani

DECLARATION BY KAZEUHIRO FUKAE UNDER 37 C.F.R. § 1.132

MS RCE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Madam:

I, Kazuhiro FUKAE, a Japanese national, having an address at c/o OTSUKA CHEMICAL CO., LTD., 463, Kagasuno, Kawauchi-cho, Tokushima-shi, Tokushima-ken 771-0193 Japan, declare that:

1. I am an inventor of the above-identified application.
2. I have studied the Office Action dated January 16, 2009
3. I have conducted the following experiments to demonstrate superiority of the present invention over the cited reference.

EXPERIMENTS

I conducted experiments on two Examples (Examples 2 and 3) and three Comparative Examples (Comparative Examples 3 to 5).

These Examples and Comparative Examples were conducted according to procedures substantially as described in Example 1 of the present application. The general conditions adopted in Examples and Comparative Examples are as follows.

Step (a)

Delipidated egg yolk (about 3g) was digested with the first Enzyme(1g) and then filtered. The filtrate was concentrated *in vacuo*, and the residual solution was purified on a gel filtration column. The oligosaccharide-containing fractions were collected, concentrated, and then lyophilized.

Step (b)

The lyophilized powder was digested with the second Enzyme (15% weight of the lyophilized powder) for 3 days, and then filtered. The filtrate was concentrated and purified on a gel filtration column. The oligosaccharide-containing fractions were collected, concentrated, and then lyophilized.

Step (c)

The lyophilized powder was dissolved in 7 times its volume of water and 5 times its volume of DMF, followed by addition of 9 equivalents of $NaHCO_3$ and 7 equivalents of Fmoc-OSu. Then, the reaction mixture was stirred for several hours (monitored by thin layer chromatography).

After the reaction, a small portion of the reaction mixture was collected and diluted with pure water. This sample was used for purification and quantitative analysis on a DEAE column.

The remaining reaction solution was added to 30 times its volume of acetone (instead of Diethyl ether in Example 1 of the present application) to induce crystallization/precipitation of the products.

Step (d)

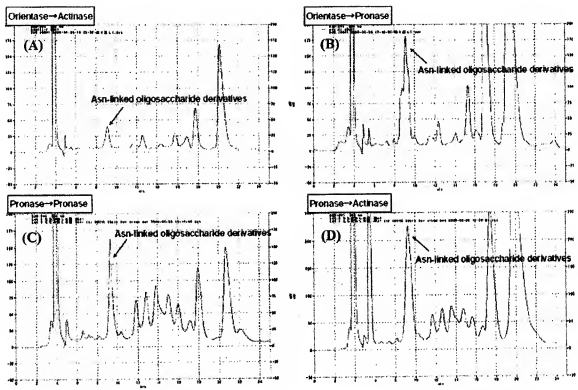
The acetone solution was filtered, and the crystals (or precipitates) were collected and then dissolved in pure water. The aqueous solution was used for purification and quantitative analysis on an ODS column.

The detailed conditions and results for each Example and Comparative Example are summarized in the table below.

RESULTS

	Example 2	Example 3	Comparative Example 3	Comparative Example 4	Comparative Example 5
the first Enzyme used in Step (a)	Orientase[*1]	Orientase[*1]	Orientase[*1]	Pronase[*3]	Pronase[*3]
the second Enzyme used in Step (b)	Actinase[*2]	Actinase[*2]	Pronase	Pronase[*3]	Actinase[*2]
delipidated egg yolk (g)	3.1138	3.0514	3.1119	3.1063	3.1106
the first Enzyme (g)	1.0053	1.0015	1.0015	1.0037	1.0069
lyophilized product (mg)	525.9	1195.5	-	613.8	999.1
the second Enzyme (mg)	80.9	184.0	81.6	94.1	151.5
the second Enzyme (kU)	80.9	184.0	5.6	6.4	151.5
lyophilized product (mg)	311.2	252.9	674.6	333.4	477.7
reaction time for Fmoc protection (hr)	26	48	26	7	5
quantification by ODS column	13.1 mg	13.4 mg	N.D.[*4]	26.3 mg	N.D.[*4]
quantification by DEAE column	13.0 mg	11.4 mg	11.2 mg	22.9 mg	27.1 mg

[*1] Orientase ONS (Hankyu Bioindustry Co., Ltd.)
[*2] Actinase E
[*3] PRONASE® Protease, Streptomyces groseus (CALBIOCHEM®)
[*4] Quantification was impossible because the product was not sufficiently separated by chromatography.



As shown in the above HPLC traces, different protease and peptidase combinations produced different product compositions, as evidenced by the different HPLC elution profiles.

Panel (A) is for a combination of orientase and actinase.
Panel (B) is for a combination of orientase and pronase.
Panel (C) is for a combination of pronase and pronase.
Panel (D) is for a combination of pronase and actinase.

The Example in panel (A) uses a process as recited in claim 1 (orientase and actinase), while the process in panel (C) is similar to the process of Inazu, except that Inazu uses pronase in a single step process.

It is clear that the product profile in panel (A) is very different from that in panel (C), indicating

that the processes are different. This observation supports the conclusion that the process recited in claim 1 is different from that of Inazu.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Executed on 15, July, 2009. K. Fukae

Kazuhiro FUKAE